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Changes in Thyroid Hormone Concentrations during Early Development and Metamorphosis of the Flounder, *Paralichthys olivaceus*

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ABSTRACT—Changes in the whole body concentrations of thyroid hormones were examined during early development and metamorphosis of the flounder (*Paralichthys olivaceus*). Thyroxine (T4) as well as triiodothyronine (T3) were detected in eggs just after fertilization; concentration of T3 was 6–7 ng/g and that of T4 was about 1 ng/g. Concentration of T3 declined gradually until hatching, decreased sharply from 5 ng/g to 0.5 ng/g within one day after hatching and became non-detectable (below 0.1 ng/g) thereafter. T4 concentration did not show marked changes until 10 days after fertilization. Until the climax of metamorphosis, T3 was undetectable and T4 concentration was less than 1 ng/g. During the climax, T4 concentration increased markedly to 10–15 ng/g, and T3 concentration increased to a detectable level (1–1.5 ng/g). After the completion of metamorphosis, T4 concentration decreased to about a half of the peak level. T3 concentration also decreased slightly. These observations were discussed in relation to the role of T4 and T3 in early development and metamorphosis of the flounder.

INTRODUCTION

Thyroid hormones are known to play an important role in amphibian metamorphosis [1]. Activation of thyroid gland was also observed during metamorphosis in the conger eel [2] and flounder [3], and treatments with thyroid hormones induced metamorphosis in these species [4–6]. Recently, a radioimmunoassay procedure was applied to estimate whole body concentrations of thyroxine (T4) during the metamorphosis of flounder larvae [7, 8], and a significant surge was observed at the climax of metamorphosis. A similar procedure was applied to examine the changes in whole body concentration of triiodothyronine (T3) during early development of chum salmon [9]. Although T3 has been shown to be more effective than T4 in inducing flounder metamorphosis [6], there is no report on the changes in T3 concentrations during the flounder metamorphosis. In this paper, we report the changes in whole body concentrations of T3 as well as T4 during early development and metamorphosis of the flounder.

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MATERIALS AND METHODS

Fertilized eggs of the flounder (*Paralichtys olivaceus*) were obtained from spawning aquarium containing several females and males, and incubated in seawater at 15°C. They hatched 4 days after fertilization. About 600 eggs or larvae were sampled daily until 8 days and on 10th day after fertilization. Larvae were offered rotifers starting from 8 days after fertilization. Another batch of larvae was obtained from a commercial source and reared in a 500 l aquarium. They were fed rotifers and brine shrimp nauplii. Twenty to 40 larvae were sampled at intervals starting from 18 days after hatching until 57 days after hatching. They were stored at −80°C until analyses. Metamorphic stages were identified by the eye migration stage and the length of the 2nd fin ray [7]. Samples of eggs and larvae until 10 days after fertilization were processed as previously described for the distribution of thyroid hormones in developing chum salmon [9]. The hormone concentrations of metamorphosing larvae were measured from one homogenate made from at least 12 individuals as follows; frozen samples were homogenized in 4 ml of methanol, and the homogenates were divided into halves, one for T4 determination and another for T3 determination, using the half volume of solutions of agents as used in previous studies [9, 10]. The least detectable concentrations of thyroid hormones were 0.1 ng/g for eggs and larvae until 10 days after fertilization and 0.4 ng/g for metamorphosing larvae.

RESULTS

Figure 1 shows the changes in T4 and T3 concentrations until 10 days after fertilization. Both T4 and T3 were detected in eggs just after fertilization; the concentration of T3 was 6.6 ng/g and that of T4 was 0.8 ng/g. T3 and T4 concentration decreased gradually toward hatching. After hatching, T3 concentration decreased sharply to about 1/10 of the level before hatching within one day, and became non-detectable thereafter. On the other hand, T4 concentration did not show such a marked change at the time of hatching, but a low level less than 1 ng/g was maintained thereafter.

As shown in Figure 2, T4 concentration during the premetamorphosis was less than 1 ng/g, and tended to increase during the prometamorphosis. T3 concentration was still non-detectable (less than 0.4 ng/g) during these periods. During the climax of metamorphosis, when the dorsal fins are being resorbed, T4 concentration increased markedly to 10–15 ng/g. T3 became detectable during the climax, but the level (1–1.5 ng/g) was still lower than the T4 level. During the postclimax of the metamorphosis, T4 concentration decreased to a half of the peak level.

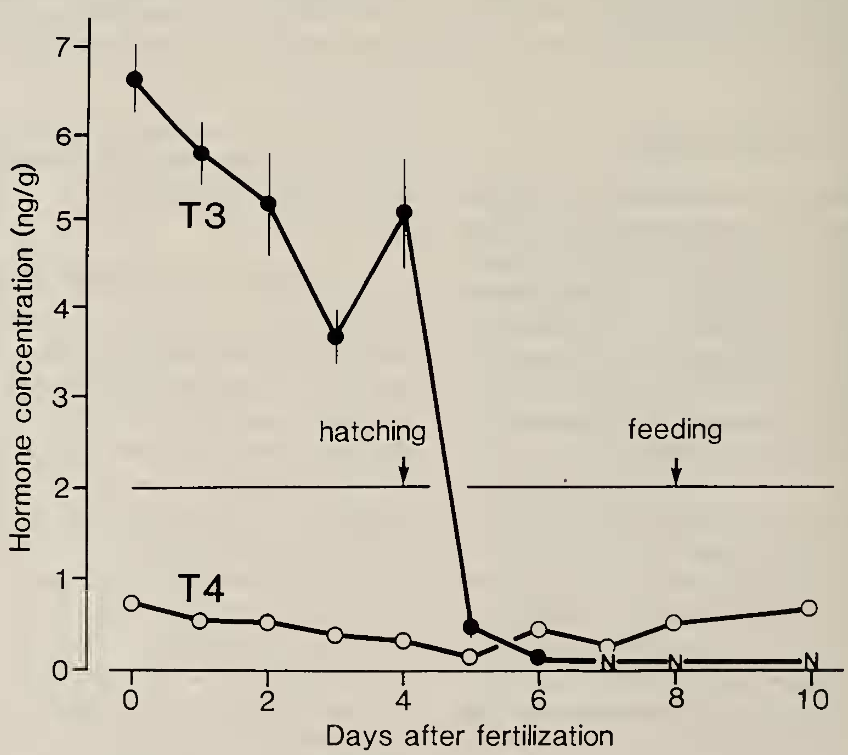

FIG. 1. Changes in T4 and T3 concentrations until 10 days after fertilization in the flounder. Vertical bars represent standard errors of the means of 3 pooled samples. In some cases, the variation was extremely small and within the size of the circle. N: non-detectable (below 0.1 ng/g).

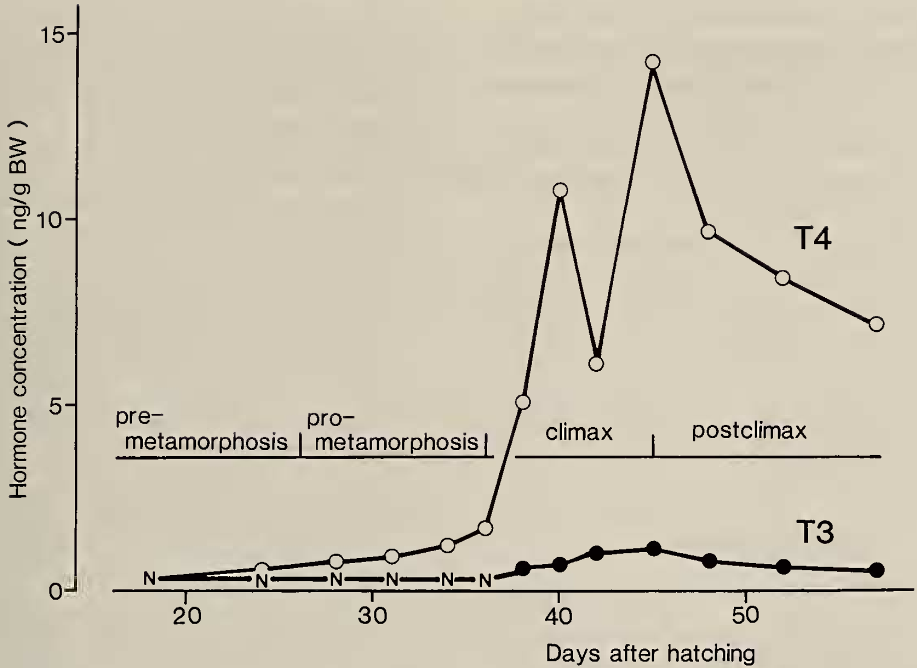

FIG. 2. Changes in T4 and T3 concentrations during metamorphosis of the flounder. Each point represents the average of the duplicate determination of one pooled sample. N: non-detectable (below 0.4 ng/g).

DISCUSSION

Significant quantities of both T4 and T3 have been detected in unfertilized or fertilized eggs of chum salmon (T4; 5–15 ng/g, T3; 4–9 ng/g) [9, 10], coho salmon (T4; 15–30 ng/g, T3; 20 ng/g) [11, 12], chinook salmon (T4; 9 ng/g, T3; 22 ng/g) [12] and striped bass (T4; 4 ng/g, T3; 5 ng/g) [13]. In the flounder eggs, T3 concentration (6.6 ng/g) was higher than T4 (0.8 ng/g) as in chinook salmon [12]. The relative quantities of T4 and T3 in fish eggs may be related to the mechanisms of utilizing thyroid hormones, such as the onset of 5'-deiodinase activity, during early developmental stages.

During the embryonic development of the flounder, whole body concentrations of both T4 and T3 decreased gradually toward hatching. After hatching, however T3 concentration decreased sharply to about 1/10 within 1 day, whereas no marked change was seen in T4 concentration. According to Fukuhara [14], a half of the yolk was resorbed within one day after hatching in the flounder larvae kept at 14.2°C, and remnant yolk was linearly resorbed to completion in succeeding 3 days. Therefore, the rate of decrease in T3 concentration in the flounder larvae just after hatching seems to be greater than that of the yolk resorption, indicating some selective absorption mechanisms of T3 from the yolk. Selective absorption of thyroid hormones during yolk resorption has also been suggested in salmonid fishes and striped bass [9–13].

Although there is no information on the onset of thyroid hormone receptors during early development of fishes, the higher concentrations of T3 than T4 and the sharp decrease in T3 concentration just after hatching seem to indicate a primary role of T3 in early development of the flounder embryo and larva. According to Eales [15], T3 seems to be the effective thyroid hormone not only in juvenile and adult fishes but also even in early developmental stages.

During the climax of the metamorphosis, T4 concentration increased markedly to 10–15 ng/g, consistent with previous observations [7, 8]. T3 concentration was non-detectable (below 0.4 ng/g) during pre- and prometamorphosis, increased to 1–1.5 ng/g at the climax when T4 surge was observed, and the same level was maintained during postclimax. According to Miwa and Inui [6], T3 was several times more effective than T4 in inducing metamorphosis in the flounder. Therefore, a small increase in T3 concentration might be enough to stimulate the metamorphosis, although it is still possible that T4, but not T3, plays important roles in the flounder metamorphosis. Distribution and development of the thyroid hormone receptors are to be studied in the "metamorphosing" organs or tissues, not only in flounders but also in fishes in general.

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